

Appl. No. 09/979,539
Amdt. dated May 6, 2004
Reply to Office Action of November 6, 2003

PATENT**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

1. (Currently amended) A polypeptide comprising a mutated antibody heavy chain variable region or light chain variable region, the polypeptide having at least 5 times higher binding affinity for an antigen bound by ~~than does a parental antibody~~ than does the parental antibody, the polypeptide having a sequence that (a) differs from the parental antibody by an amino acid substitution of at least one amino acid in a complementarity determining region (CDR), the amino acid in the parental antibody being encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T, and (b) does not differ from the parental antibody in the amino acids in the CDR with respect to amino acids encoded by a codon that does not belong to one of these two hot spot motifs.

2. (Original) The polypeptide of claim 1 wherein the substitution occurs in CDR3 of a light chain variable region.

3. (Original) The polypeptide of claim 1 wherein the substitution occurs in CDR3 of a heavy chain variable region.

4. (Original) The polypeptide of claim 1 wherein the substitution occurs in CDR1 or CDR2 of a light chain variable region.

5. (Original) The polypeptide of claim 1 wherein the substitution occurs in CDR1 or CDR2 of a heavy chain variable region.

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6. (Original) The polypeptide of claim 2 wherein the antigen is mesothelin, the parental antibody is antimesothelin antibody SS and the polypeptide has a sequence that differs from antibody SS by an amino acid substitution of at least one amino acid selected from S92, G93 and Y94.

7. (Original) The polypeptide of claim 6 wherein the substitutions are selected from G93K-Y94H (SS1); S92G-G93F-Y94N (D8) and S92G-G93S-Y94H (C10).

8. (Original) The polypeptide of claim 1, wherein said polypeptide is a scFv.

9. (Original) The polypeptide of claim 6, wherein said polypeptide is a scFv.

10. (Original) The polypeptide of claim 1, wherein said polypeptide is a dsFv, a Fab, or a F(ab')₂.

11. (Original) The polypeptide of claim 6, wherein said polypeptide is a dsFv, a Fab, or a F(ab')₂.

12. (Original) The polypeptide of claim 1 further comprising a therapeutic moiety or a detectable label.

13. (Original) The polypeptide of claim 12, wherein the therapeutic moiety is a toxic moiety.

14. (Original) The polypeptide of claim 13, wherein the toxic moiety is a *Pseudomonas* exotoxin or a cytotoxic fragment thereof.

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15. (Original) The polypeptide of claim 14, wherein the toxic moiety is a cytotoxic fragment, which is PE38.
16. (Original) The polypeptide of claim 13, wherein the toxic moiety is selected from the group consisting of diphtheria toxin or a cytotoxic fragment thereof, saporin or a cytotoxic fragment thereof, pokeweed antiviral toxin or a cytotoxic fragment thereof, ricin or a cytotoxic fragment thereof, and bryodin I or a cytotoxic fragment thereof.
17. (Original) The polypeptide of claim 6, further comprising a therapeutic moiety or a detectable label.
18. (Original) The polypeptide of claim 17, wherein the therapeutic moiety is a toxic moiety.
19. (Original) The polypeptide of claim 18, wherein the toxic moiety is a *Pseudomonas* exotoxin or a cytotoxic fragment thereof.
20. (Original) The polypeptide of claim 19, wherein the toxic moiety is a cytotoxic fragment, selected from the group consisting of PE35, PE38, and PE40.
21. (Currently amended) The polypeptide of claim 1, ~~further comprising~~ expressed in conjunction with a surface protein of a bacteriophage.
22. (Currently amended) A polypeptide which has a binding affinity for mesothelin at least three times that of antimesothelin antibody SS, which polypeptide has complementarity determining regions (CDRs), having a sequence which differed differs from that of antibody SS by an amino acid substitution in CDR3 of a light chain variable region of at least L96T (E4).

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23. (Original) The polypeptide of claim 22, further comprising a therapeutic moiety or a detectable label.

24. (Original) The polypeptide of claim 23, wherein the therapeutic moiety is a toxic moiety.

25. (Original) The polypeptide of claim 24, wherein the toxic moiety is a *Pseudomonas* exotoxin or a cytotoxic fragment thereof.

26. (Original) The polypeptide of claim 25, wherein the toxic moiety is a cytotoxic fragment, selected from the group consisting of PE35, PE38, and PE40.

27. (Currently amended) A nucleic acid molecule encoding a polypeptide comprising a mutated antibody heavy chain variable region or light chain variable region, the polypeptide having at least 5 times higher binding affinity for an antigen bound by than does a parental antibody than does the parental antibody, the polypeptide having a sequence that (a) differs from a parental antibody by an amino acid substitution of at least one amino acid in a complementarity determining region (CDR), the amino acid in the parental antibody being encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T, and (b) does not differ from the parental antibody in the amino acids within the CDR with respect to amino acids not encoded by a codon with a nucleotide belonging to a hot spot motif selected from AGY or RGYW.

28. (Original) The nucleic acid molecule of claim 27, wherein the antigen is mesothelin, the parental antibody is antimesothelin antibody SS and the polypeptide has a sequence that differs from antibody SS by an amino acid substitution of at least one amino acid selected from S92, G93 and Y94.

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29. (Original) The nucleic acid molecule of claim 27, wherein the substitutions are selected from G93K-Y94H (SS1); S92G-G93F-Y94N (D8) and S92G-G93S-Y94H (C10).

30. (Original) A nucleic acid molecule encoding a polypeptide which has a binding affinity for mesothelin at least three times that of antimesothelin antibody SS, which polypeptide has a sequence which differed from antibody SS by an amino acid substitution in CDR3 of a light chain variable region of at least L96T (E4).

31. (Original) An expression cassette comprising a promoter operably linked to a nucleic acid molecule of claim 27.

32. (Original) An expression cassette comprising a promoter operably linked to a nucleic acid molecule of claim 28.

33. (Currently amended) A method of killing a malignant cell bearing an antigen, comprising contacting the cell with an immunotoxin comprising a toxic moiety and a targeting moiety, the targeting moiety comprising a polypeptide comprising a mutated antibody heavy chain variable region or light chain variable region, the polypeptide having at least 5 times higher binding affinity for an antigen bound by than does a parental antibody than does the parental antibody, the polypeptide having a sequence that (a) differs from the parental antibody by an amino acid substitution of at least one amino acid in a complementarity determining region (CDR), the amino acid in the parental antibody being encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T, and (b) does not differ from the parental antibody in the sequence of amino acids within the CDR with respect to amino acids not encoded by a codon with a nucleotide belonging to a hot spot motif selected from AGY or RGYW.

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34. (Original) The method of claim 33, wherein the antigen is mesothelin.

35. (Currently amended) The method of claim 34, wherein the targeting moiety ~~is selected from the group consisting of SS1, D8, and C10~~ has a sequence that varies from SS antibody by having substitutions selected from the group consisting of G93K-Y94H (SS1); S92G-G93F-Y94N (D8) and S92G-G93S-Y94H (C10).

36. (Original) The method of claim 35, wherein said toxic moiety is a *Pseudomonas* exotoxin or cytotoxic fragment thereof.

37. (Original) The method of claim 36, wherein the toxic moiety is a cytotoxic fragment, selected from the group consisting of PE35, PE38, and PE40.

38. (Original) A method of killing a malignant cell bearing an antigen, comprising contacting the cell with an immunotoxin comprising a toxic moiety and a targeting moiety, wherein the targeting moiety is antibody E4.

39. (Original) The method of claim 38, wherein said toxic moiety is a *Pseudomonas* exotoxin or cytotoxic fragment thereof.

40. (Currently amended) The method of claim 38, wherein the toxic moiety is a cytotoxic fragment, selected from the group consisting of PE35, PE38, and PE40.

41. (Withdrawn) A method of identifying a polypeptide which has a higher affinity for a target antigen than does a parental antibody, comprising
(a) contacting a polypeptide of claim 1 with the target antigen under conditions appropriate for specific binding between an antibody and the target antigen,

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(b) eluting the polypeptide under conditions which remove any antibody or fragment thereof which have not bound to the target antigen with an affinity higher than that of the parental antibody or fragment thereof, and

(c) determining whether the polypeptide is bound to the antigen, whereby binding identifies the polypeptide as having a higher affinity for the target than does the parental antibody.

42. (Withdrawn) A method of making a library of nucleic acids encoding mutated antibody variable domains comprising:

a) providing a nucleic acid molecule encoding an amino acid sequence of a V_H or a V_L domain of a parental antibody, the nucleic acid molecule comprising at least one parental hot spot codon comprising at least one nucleotide within a hot spot motif;

b) generating a plurality of mutated nucleic acid molecules encoding mutated amino acid sequences that differ from the parental amino acid sequence wherein each mutated nucleic acid sequence comprises at least one mutated codon different than a parental hot spot codon encoding an amino acid, the mutated codon encoding an amino acid different than the amino acid encoded by the parental hot spot codon.

43. (Withdrawn) The method of claim 42 wherein the plurality of mutated nucleic acid molecules contains at least 19 members, wherein each of the 19 members encodes an amino acid sequence in which the amino acid encoded by the parental hot spot codon is replaced by a different natural amino acid.

44. (Withdrawn) The method of claim 42, wherein the plurality of mutated nucleic acid molecules comprises mutated codons different than at least two parental hot spot codons encoding amino acids, each of the mutated codons encoding an amino acid different than the amino acid encoded by the parental hot spot codon.

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45. (Withdrawn) The method of claim 42, wherein the plurality of mutated nucleic acid molecules comprises at least 399 members, each of which members encodes an amino acid sequence in which the amino acids encoded by the parental hot spot codons is replaced by a different natural amino acid.

46. (Withdrawn) The method of claim 42, further wherein the parental antibody is of a class of antibodies having at least one conserved amino acid encoded by a codon, wherein the codon or codons encoding the conserved amino acids are not mutated.

47. (Withdrawn) The method of claim 42, wherein the hot spot motif is selected from the group consisting of AGCA, AGTT, AGCT, AGTA, GGCA, GGTT, GGCT, GGTA, AGC, and AGT.

48. (Withdrawn) The method of claim 42, wherein the mutated nucleic acid molecule comprises at least one mutated codon within a portion of the V_H or the V_L domain comprising a CDR.

49. (Withdrawn) The method of claim 48, wherein the CDR is the CDR3 of the V_H domain.

50. (Withdrawn) The method of claim 48, wherein the CDR is the CDR3 of the V_L domain.

51. (New) A polypeptide comprising a mutated antibody heavy chain variable region or light chain variable region, the polypeptide having at least 5 times higher binding affinity for an antigen bound by SS antibody than does SS antibody, the polypeptide having a sequence that differs from SS antibody by an amino acid substitution of at least one amino acid in a complementarity determining region (CDR), the amino acid in the SS antibody being

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encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T.

52. (New) The polypeptide of claim 51 wherein the substitution occurs in CDR3, CDR2, or CDR1 of a light chain variable region.

53. (New) The polypeptide of claim 51 wherein the substitution occurs in CDR3, CDR2, or CDR1 of a heavy chain variable region.

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~~56.~~ (New) The polypeptide of claim 51 wherein the polypeptide has a sequence that differs from antibody SS by an amino acid substitution of at least one amino acid selected from S92, G93 and Y94.

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~~57.~~ (New) The polypeptide of claim ⁵⁴~~56~~, wherein the substitutions are selected from G93K-Y94H (SS1); S92G-G93F-Y94N (D8) and S92G-G93S-Y94H (C10).

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~~58.~~ (New) The polypeptide of claim 51, wherein said polypeptide is a scFv, dsFv, a Fab, or a F(ab')₂.

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~~59.~~ (New) The polypeptide of claim 51, conjugated to or fused to a therapeutic moiety or a detectable label.

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~~60.~~ (New) The polypeptide of claim ⁵⁷~~59~~, wherein the therapeutic moiety is a toxic moiety.

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~~61.~~ (New) The polypeptide of claim ⁵⁸~~60~~, wherein the toxic moiety is a *Pseudomonas* exotoxin or a cytotoxic fragment, mutant, or cytotoxic fragment of a mutant thereof.

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~~62.~~ (New) A nucleic acid molecule encoding a polypeptide comprising a mutated antibody heavy chain variable region or light chain variable region, the polypeptide having at least 5 times higher binding affinity for mesothelin than does antibody SS, the polypeptide having a sequence that differs from the SS antibody by an amino acid substitution of at least one amino acid in a complementarity determining region (CDR), the amino acid in the SS antibody being encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T.

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~~63.~~ (New) The nucleic acid molecule of claim ~~62~~, wherein the polypeptide has a sequence that differs from antibody SS by an amino acid substitution of at least one amino acid selected from S92, G93 and Y94.

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~~64.~~ (New) The nucleic acid molecule of claim ~~62~~, wherein the substitutions are selected from G93K-Y94H (SS1); S92G-G93F-Y94N (D8) and S92G-G93S-Y94H (C10).

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~~65.~~ (New) An expression cassette comprising a nucleic acid molecule of claim 27 operably linked to a promoter.

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~~66.~~ (New) A method of killing a malignant cell bearing mesothelin antigen, comprising contacting the cell with an immunotoxin comprising a toxic moiety and a targeting moiety, the targeting moiety comprising a polypeptide comprising a mutated antibody heavy chain variable region or light chain variable region, the polypeptide having at least 5 times higher binding affinity for mesothelin than does SS antibody, the polypeptide having a sequence that differs from SS antibody by an amino acid substitution of at least one amino acid in a complementarity determining region (CDR), the amino acid in the SS antibody being encoded by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and W is A or T.

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~~67.~~ (New) The method of claim ⁶⁶~~66~~, wherein the targeting moiety has a sequence that varies from SS antibody by having substitutions selected from the group consisting of G93K-Y94H (SS1); S92G-G93F-Y94N (D8) and S92G-G93S-Y94H (C10).

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~~68.~~ (New) The method of claim ⁶⁶~~66~~, wherein said toxic moiety is a *Pseudomonas* exotoxin or cytotoxic fragment or mutant thereof.